

Analysis of Diatom Blooms Using DNA Fingerprints

E. Virginia Armbrust
University of Washington
School of Oceanography, Box 357940
Seattle, WA 98195
phone: (206) 616-1783 fax: (206) 685-6651 email: armbrust@ocean.washington.edu

Grant Number: N00014-99-1-0060
<http://www.onr.navy.mil/oas/onrpgahj.htm>

LONG-TERM GOALS

My long-term goals are to understand the interaction between the biological and environmental factors that dictate the timing and magnitude of diatom blooms. I am particularly interested in how species behavior is coupled to environmental conditions and how the extent of genetic and physiological diversity within a population influences its future ability to bloom.

OBJECTIVES

The premise that guides my research is that phytoplankton community dynamics reflect a complicated interaction between environmental variability and the inherent genetic and physiological variation present within individual species of phytoplankton. My goal is to determine how genetic and physiological diversity is maintained within individual species of diatoms and how this diversity is shaped by different environments and on different time scales. Ultimately, this should allow me to determine how population diversity is coupled to future blooming capabilities.

APPROACH

Our goal is to determine how genetic and physiological diversity is shaped by the environment in order to better understand diatom bloom dynamics. Our studies focus on the unicellular centric diatom *Ditylum brightwellii* because of the importance of this diatom in coastal waters and because of the ease of identifying it in mixed populations. We examine genetic diversity within diatom populations by utilizing high-throughput DNA fingerprinting techniques. We base our DNA fingerprints on highly repetitive regions of DNA known as microsatellites. The length of any given repetitive region can vary dramatically between individuals and so can be used to define individuals. We determine genetic diversity within *D. brightwellii* populations by first isolating individual cells into about 1 ml of media (in a 48-well plate) and then allowing the cells to divide asexually (1-2 weeks). Each isolate can be thought of as a different individual composed of many genetically identical cells. A subset of isolates is maintained in culture for physiological studies. DNA is extracted from all isolates and polymerase chain reaction (PCR) is used to amplify specific microsatellites. The length of a microsatellite defines the allele size; the composite of allele sizes at different microsatellite loci defines the DNA fingerprint for an individual; the combination of individual fingerprints defines a population. We use the population-based information to determine whether genetically defined populations co-vary with different environmental conditions.

Report Documentation Page				Form Approved OMB No. 0704-0188	
Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.					
1. REPORT DATE 30 SEP 2004		2. REPORT TYPE		3. DATES COVERED 00-00-2004 to 00-00-2004	
4. TITLE AND SUBTITLE Analysis of Diatom Blooms Using DNA Fingerprints				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Washington,,School of Oceanography, Box 35794,,Seattle,,WA,98195				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT My long-term goals are to understand the interaction between the biological and environmental factors that dictate the timing and magnitude of diatom blooms. I am particularly interested in how species behavior is coupled to environmental conditions and how the extent of genetic and physiological diversity within a population influences its future ability to bloom.					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT Same as Report (SAR)	18. NUMBER OF PAGES 6	19a. NAME OF RESPONSIBLE PERSON
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified			

WORK COMPLETED

Over the past year, we have focused on analyzing the DNA fingerprints of isolates collected during two field studies Puget Sound, WA; in conjunction with the DNA data, we also have CTD and nutrient data. In the first study, *D. brightwellii* isolates were collected from a single site in Dabob Bay over the course of 11 days during a spring bloom (March/April). One thousand eight isolates were collected during this study, with 820 surviving as unialgal isolates for DNA isolation and genotyping. We have completed analysis of this data and are the process of writing a manuscript on this work. The second field study was conducted in collaboration with the Washington State Department of Ecology and consists of samples collected at four sites within Puget Sound and at one site in the Strait of Juan de Fuca near the entrance to Puget Sound. Samples were collected over the course of 6 months from February to July. The analysis of this data is ongoing. Recently, we have begun a series of experiments to determine whether physiological factors distinguish populations isolated from Puget Sound and the Strait of Juan de Fuca.

RESULTS

The work described below has been conducted by me, my graduate student (and now postdoc), Tatiana Rynearson, and two undergraduates, Chuck Lausche and Rhonda Marohl. One paper has been submitted and is under revision for *Journal of Phycology*. A second manuscript is currently in preparation.

In a previous study, we described a population of *D. brightwellii* cells within Puget Sound that was repeatedly sampled over 2.5 years. Importantly, this population was physiologically and genetically diverged from *D. brightwellii* populations detected within Strait of Juan de Fuca waters. We have hypothesized that two distinct, but interacting factors facilitate maintenance of genetic differentiation between Sound and Strait populations despite regular mixing of the two bodies of water. First, approximately 60% of out-flowing water is refluxed back into the Sound at depth upon each tidal cycle, which retains Puget Sound-derived *D. brightwellii* cells within the Sound. Presumably this recirculation allows sufficient time for cells to adapt to their environment. No obvious recirculation mechanism exists for the Strait. Second, we hypothesize that *D. brightwellii* cells from the Strait are unable to reproduce in Puget Sound, despite the fact that Strait cells display faster maximum growth rates under optimal conditions in the laboratory.

Although there are many possible factors that differ between the waters of Puget Sound and the Strait, we began our studies by examining whether isolates from the Strait and the Sound display different responses to prolonged periods of darkness since the denser Strait of Juan de Fuca water mass will immediately sink to depth, carrying cells with it. We hypothesized that an indigenous Puget Sound population, continuously refluxed back into the Sound, would be better adapted to cycling between extended periods in the dark at depth and extended periods in the light at the surface. To test this possibility, two isolates each from the Sound and the Strait populations were maintained in exponential growth under identical conditions (16:8 light:dark cycle of $66 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and replicate flasks were placed in the dark for either 1, 2 or 3 weeks before a return to the same light conditions as before the experiment began. Cell counts for each flask were monitored over time. After 1 week in the dark, isolates from the Strait and the Sound were able to quickly begin cell division upon a return to the light, each displaying growth rates that were indistinguishable from one another. After two weeks in the dark, the post-dark growth rate of the Puget Sound isolate remained unchanged, but the growth rate

of the Strait isolate was significantly reduced. After 3 weeks in the dark, the post-dark growth rate of the Puget Sound isolate slowed by about 3.5-fold, whereas the Strait isolate slowed by at least 20-fold such that it was essentially unable to re-initiate cell division. Although we only have results for two isolates each from the two populations, the data is quite provocative and suggests that Strait-derived *D. brightwellii* cells may not recover as quickly from the extended dark periods they would experience upon entry into Puget Sound.

The next study focused on the *D. brightwellii* population detected within Puget Sound. In spring of 00, we were able to follow a bloom of *D. brightwellii* over the course of 11 days in Dabob Bay. For 10 of the 11 days, 96 individual *D. brightwellii* cells were isolated from surface waters; on the first day of sampling, 48 cells were isolated. In addition, samples were fixed each day for cell counts and CTD profiles were conducted. On day 5, a windstorm occurred that deepened the mixed layer and immediately decreased cell concentration in surface waters by about half. Sampling continued until cell numbers declined to pre-bloom level.

We have now analyzed all the data collected during this study and are in the process of writing it up for publication. DNA from a total of 820 single cell isolates was extracted and genotyped. Three-locus genotypes were obtained from 607 isolates. As expected, analysis of allele distributions indicated that the same population previously identified in Puget Sound was sampled each day of the bloom. What was surprising was that diversity did not decrease during the course of the bloom. About 80% (497) of the sampled isolates were genetically distinct. This is a remarkable finding since, during a bloom, asexual reproduction generates multiple genetically identical copies of individual cells. We have hypothesized that high levels of diversity are maintained in diatom populations because the environment changes frequently enough that no single clonal lineage can become numerically dominant (Rynearson et al. 2000).

Interestingly, we did detect two different DNA fingerprints seven times during course of the bloom. Probability analyses were used to determine that individuals with identical three-locus fingerprints could be considered genetically identical clones. Thus, repeated identification of the same fingerprint indicated repeated sampling of a given clonal lineage. Even more remarkably, one DNA fingerprint detected twice on 25 March and one time each on 30 March and 1 April had been previously detected once in November of 1997 in Hood Canal, which suggests that this is a particularly successful clonal lineage that has persisted in Hood Canal for at least 2.5 years. The re-sampling of individual clones also allowed us to also use capture/re-capture statistics to predict the total number of clones in the population. We calculated that the population originating within Puget Sound is composed of a minimum of ~2500 genetically distinct clones.

We also compared the observed frequency of these repeatedly-sampled clones before and after the windstorm and found that there was a significant change in re-sampling frequencies. We interpret this data in two ways. First, it does appear that some clonal lineages have a growth advantage over other clonal lineages such that particular clones are more common in a population than others. Second, none of these clones can come to completely dominate a population because small-scale environmental variations like wind-storms, reshuffle which clones possess a growth advantage under any given set of conditions, allowing diversity to be maintained within a population (Fig. 1).

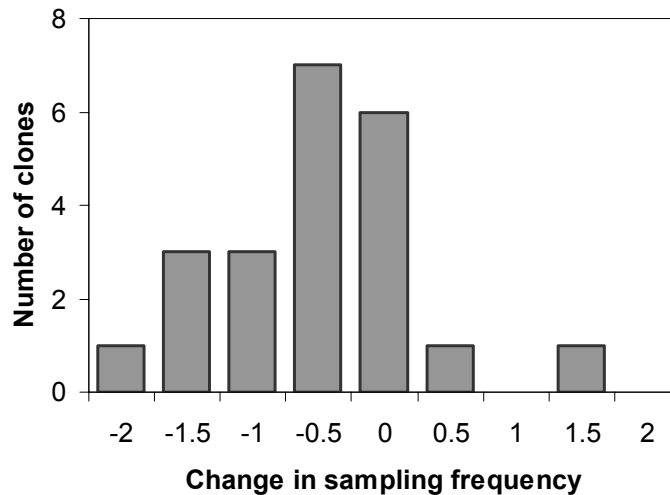


Figure 1. Change in frequency of 22 clonal lineages sampled both before and after the storm. Clonal lineages sampled as frequently before the storm as after display a change in sampling frequency of zero. Clonal lineages sampled more frequently before than after the storm are negative and those sampled more frequently after the storm are positive. More clones were sampled before the storm than after.

We next asked whether additional populations of *D. brightwellii* inhabited Puget Sound. We expanded our sampling scheme to span additional months and to include additional sites. In collaboration with Dr. Jan Newton and the Washington State Department of Ecology, we obtained monthly water samples from February through June of 2000 at two sites within Hood Canal (North, South), 2 sites within the Main Basin (North, South) and 1 site just outside Puget Sound within the Strait of Juan de Fuca. Up to 96 individual cells were isolated from each water sample in which *D. brightwellii* could be found. Cells were detected and isolated between February and April in Hood Canal and from April to June in the Main Basin; cells were found in the Strait of Juan de Fuca throughout the sampling period. In total, 11 water samples contained sufficient *D. brightwellii* cells for DNA fingerprinting analysis. CTD data and nutrient profiles are available for each sample, and water was fixed for *D. brightwellii* cell counts and determination of cell size distributions. Significant numbers of *D. brightwellii* cells were present in Hood Canal from February to April and in the Main Basin from April to June. We have focused our initial analyses on those samples with the most cells.

For February and March, only the samples from Hood Canal have been DNA fingerprinted and thus far we have only detected the same resident population previously detected in Puget Sound. In April, all Puget Sound sites have been analyzed but again, only this same population is detected. In May and June, significant numbers of *D. brightwellii* were only found in the Main Basin. Interestingly, in these samples, what we had initially believed to be the only resident Puget Sound population was replaced by a new, significantly diverged population based on both F_{st} values and cell size. This new May/June population has a significantly different allele distribution and the cells are significantly larger in size than the cells detected in Hood Canal just one month earlier. Moreover, this new population is also significantly diverged from the two Strait of Juan de Fuca populations detected in 1998. Thus, it appears that within Puget Sound there is an early-blooming and a late-blooming population of *D. brightwellii*. Previous to this study, we had repeatedly detected this early-blooming population. We cannot yet definitively determine the source of origin for this newly detected population within Puget

Sound. However, it seems probable that it originated from within the Sound, perhaps from the South Basin, which empties into the Main Basin.

IMPACT/APPLICATION

We have shown that eukaryotic microbes can adapt to local environmental conditions and that it is possible to define resident members of a community, even those members that are wholly planktonic. It appears that in the ocean, weak physical retention of cells in combination with differential selection can maintain uniquely adapted populations of rapidly dividing microbes. We should now be able to examine in more detail those physiological characteristics that are under selection and we can examine the bloom dynamics of these resident populations under changing environmental conditions. This new way of thinking about physical and genetic partitioning in the marine environment has ramifications for a broad range of research arenas, from management of coastal regions to theories of speciation.

RELATED PROJECTS

We have an ongoing collaboration with Peter Franks from Scripps Institution of Oceanography to develop microsatellite-based approaches to understand dinoflagellate bloom dynamics. We have now identified about 50 microsatellites in *Prorocentrum micans* and have so far, designed PCR primers against ~10 loci and have identified at least one promising primer set.

PUBLICATIONS

Rynearson, T. A. and E. V. Armbrust. 2003. Genetically and physiologically distinct populations of the planktonic diatom *Ditylum brightwellii* maintained in a dynamic marine environment. In revision, *Journal of Phycology*.

Rynearson, T.A. and E.V. Armbrust. 2003. Population differentiation in a marine phytoplankton: dispersal vs. selection. Annual Joint Meeting of the Society for the Study of Evolution, Society of Systematic Biologists, and American Society of Naturalists.

Rynearson, T. A. and E. V. Armbrust. 2003. Genetic diversity and selection during a spring diatom bloom. American Society of Limnology and Oceanography annual meeting.